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Matthew Madsen — Signature

#19

PATENT 3/19/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Stanislaw R. Burzynski

Serial No.: 09/603,320

Filed: June 26, 2000

For: PHENYLACETIC ACID COMPOSITIONS FOR TREATING OR PREVENTING HYPERCHOLESTEROLEMIA

Confirmation No.: 3169

Group Art Unit: 1617

Examiner: BAHAR, M.

Atty. Dkt. No.: 10379.0046.NPUS00

APPEAL BRIEF

BOX AF
Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicant hereby submits an original and two copies of this Appeal Brief to the Board of Patent Appeals and Interferences in response to the final Office Action dated August 14, 2001.

The Commissioner is authorized to charge the fee of **320.00**, which is the fee for filing a brief in support of an appeal, to Deposit Account No. 01-2508/10379.0046.NPUS00. Should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, the Commissioner is authorized to deduct said fees from Deposit Account No. 01-2508/**10379.0046.NPUS00**.

Please date stamp and return the enclosed postcard to evidence receipt of this document.

I. REAL PARTY OF INTEREST

Applicant, Stanislaw R. Burzynski, is the real party of interest.

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II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or Interferences, known to the Appellant or Appellant's legal representatives, which directly affect, are directly affected by, or have any bearing on the Board's decision in the instant appeal.

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III. STATUS OF THE CLAIMS

Claims 5-7, 9-15, 17, and 18 were cancelled by Amendment, in accordance with 37 C.F.R. §1.121(c), in the response, filed April 24, 2001, in reply to the Office Action dated January 30, 2001. Claim 1 was amended and new claims 19-23 were added by that same Amendment.

Claim 16 is withdrawn from consideration as allegedly being drawn to a non-elected invention.

Consequently, claims 1-4, 8, and 19-23 are on appeal.

***

IV. STATUS OF AMENDMENTS

No amendment was filed subsequent to the final rejection. Claims 1-4, 8, and 19-23 are on appeal and claims 1-4, 8, 16, and 19-23, as currently pending, appear in Appendix A.

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V. SUMMARY OF THE INVENTION

The invention claimed is drawn to a method for treating a patient afflicted with hypercholesterolemia or hypertriglyceridemia by administering, to the patient a composition comprising a therapeutically-effective amount of 3-phenylacetlamino-2,6-piperidinedione and/or derivatives thereof.

The related art describes the *in vitro* use of compounds such as 3-phenylacetlamino-2,6-piperidinedione, and/or its hydrolysis products, to inhibit a single enzyme known to catalyze a reaction in the cholesterol biosynthetic pathway. (*See*, instant Specification, page 3, lines 18-22). Nevertheless, there was no suggestion that such compounds would prevent cholesterol synthesis *in vivo*, or otherwise cause a reduction serum cholesterol levels *in vivo*.. In contrast, the instantly claimed methods provide for dramatic reductions in the serum cholesterol and triglyceride levels of patients treated therewith. (*See,* the example in the Specification at page 16, line 26 through page 17, line 13.)

***

VI. ISSUES ON APPEAL

The issues on appeal are:

- Whether it is proper to reject claims 1-4, 8, and 19-23 under 35 U.S.C. § 103(a) as being unpatentable over alleged applicant admissions in the specification in view of U.S. Pat. No. 5,238,947 (hereinafter referred to as "Hendry *et al.*"), even though the cited art provides no evidence or suggestion for the presently claimed methods.

- Whether, if read in context, the sentence in the specification at page 3, lines 18-23 constitutes an admission that is was known in the art that 3-phenylacetylamino-2,6-piperidinedione and its derivatives "may lower cholesterol levels", even though the subsequent sentence clearly conveys that the phrase was purely speculative.
- Whether the fact that a compound is known to inhibit, *in vitro*, a single enzymatic step in the cholesterol biosynthetic pathway renders it obvious, as defined by 35 U.S.C. § 103(a), that the compound is effective to treat hypercholesterolemia and/or hypertriglyceridemia in affected patients, even though there is no teaching or suggestion in the prior art which provides *in vivo* evidence that the compound has any effect on serum cholesterol or triglyceride levels.

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VII. GROUPING OF THE CLAIMS

The patentability of claims 2-4, 8, and 19-23 stand or fall with that of independent claim 1.

***

VIII. ARGUMENT

A. Rejection of claims 1-4, 8, and 19-23, under 35 U.S.C. § 103(a), over alleged admissions of Applicant in view of Hendry et al. (U.S. Patent 8,238,947).

The Specification for the instant application recites *inter alia* that:

> [i]t has been known for some time that compounds such as 3-phenylacetylamino-2,6-piperidinedione and its hydrolysis products, such as phenylacetic acid, and salts, precursors, and analogs thereof (together, "3-phenylacetylamino-2,6-piperidinedione and its derivatives"), can block the formation of iso-pentenylpyrophosphate from 5-pyrophosphomevalonate, a reaction in the pathway of cholesterol biosynthesis; as a result, these compounds may lower serum cholesterol levels. Therefore, it was desirable to determine which, if any, of 3-phenylacetylamino-2,6-piperidinedione and its derivatives can lower serum cholesterol levels, and thus can form the basis of a pharmaceutical composition

> useful in treating or prevent hypercholesterolemia. Derivatives of 3-phenylacetylamino-2,6-piperidinedione that exhibit such activity are disclosed herein.

See, Specification page 3, lines 18-28, (emphasis added). This passage clearly indicates Applicant's position that, there was no teaching or suggestion, in the prior art, that any of the recited compounds would be effective to treat or prevent hypercholesterolemia, although it was known that derivatives of 3-phenylacetylamino-2,6-piperidinedione could inhibit a single enzymatic step in the cholesterol biosynthetic pathway.

In contrast, it is the Examiner's position that the passage above constitutes an admission that it was known in the prior art that the recited compounds "may lower serum cholesterol levels." Furthermore, the Examiner contends that this alleged admission, when taken in view of Hendry *et al.* renders the appealed claims obvious within the meaning of 35 U.S.C. § 103(a). Specifically, the Examiner argues that:

> One of ordinary skill in the art would have been motivated to combine these teachings in order to employ phenylacetylglutamine sodium in a method of treating or inhibiting hypercholesterolemia because phenylacetylamino-2,6-piperidinedione [sic] is known to be hydrolyzed in a host in vivo to produce phenylacetylglutamine. Therefore, similar antihypercholesterolemic effects in the host (i.e. affected patient) for both compounds would reasonably be expected. Given the current state of the art, determining the active ingredient dosage level is well within the Skilled Artisan's purview and the benefits of achieving such maximization obvious, to said Skilled Artisan. The optimization of amounts of active ingredients to be employed is considered within the skill of the artisan.

See, August 14, 2001 Office Action, final paragraph of page 4. Applicant traverses.

The Court of Appeals of the Federal Circuit has held that:

> [w]here claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under § 103 requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. *See In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). Both the suggestion and the

> reasonable expectation of success must be found in the prior art, not in the applicant's disclosure. *Id.*

In re Vaeck, 947 F.2d 488, 493, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). Applicant contends that, contrary to the standard established in *Vaeck*, the cited art provides no reasonable expectation for the success of the claimed methods. Moreover, even if the Examiner's position--that the passage recited *supra* constitutes and admission that it was known in the prior art that the claimed compounds may be useful for treating hypercholesterolemia--were conceded as being accurate (a position unequivocally disputed by Applicant), the combination of this alleged admission taken together with the teachings of Hendry *et al.* simply does not provide a reasonable expectation of success for the instantly claimed invention. The mere fact that a compound is known to inhibit the *in vitro*, or even *in vivo* function of a single enzyme known to be involved in the highly complex and regulated cholesterol biosynthetic pathway, cannot possibly provide proof or a reasonable expectation that using similar compounds would lower serum cholesterol and/or triglyceride levels. In support of this position Applicant offers the following excerpt from a respected biochemistry textbook:

> [h]igh blood cholesterol **(hypercholesterolemia)**, which results from the overproduction and/or underutilization of LDL [low density lipoprotein], is known to be caused by two metabolic irregularities: (1) the genetic disease familial **hypercholesterolemia (FH)**; or (2) the consumption of a high cholesterol diet. FH is a dominant genetic defect that results in a deficiency of functional LDL receptors . . . .

DONALD VOET & JUDITH G. VOET, BIOCHEMISTRY, SEC. 23-6: CHOLESTEROL METABOLISM 655-656 (John Wiley & Sons, New York 1990) (emphasis in original). A copy of the title page and cited text pages is attached hereto as Appendix B.

In view of the manifold factors which regulate serum cholesterol levels, which were well known to those of ordinary skill in the art at the time the application was filed, Applicant asserts that the combination of art cited by the Examiner fails to provide a reasonable expectation of

success. As indicated to the Examiner in Applicant's response, filed April 24, 2001, Applicant believes that, at worst (with regard to the patentability of the claims), the combination cited by the Examiner provides a "motivation to try" or to conduct further experimentation. *See*, pages 6 and 7 of Applicant's April 24, 2001 response. Furthermore, the courts have been highly critical of the "obvious to try" standard. For example, in *In re Lindell* the court emphasized that:

> we have criticized the "obvious to try" test on several occasions . . . .
>
> Furthermore, applications of the "obvious to try" test would often deny patent protection to inventions growing out of well-planned research which is, of course, guided into those areas in which success is deemed most likely. These are, perhaps, the obvious areas to try. But resulting inventions are not necessarily obvious. Serendipity is not a prerequisite to patentability. Our view is that "obvious to try" is not a sufficiently discriminatory test.

In re Lindell, 155 U.S.P.Q. 521, 523 (C.C.P.A. 1967). Additionally, the Court of Appeals of the Federal Circuit has described what is meant by "obvious to try" when it stated that:

> [a]n "obvious-to-try" situation exists when a general disclosure may pique the scientist's curiosity, such that further investigation might be done as a result of the disclosure, but the disclosure itself does not contain a sufficient teaching of how to obtain the desired result, or that the claimed result would be obtained if certain directions were pursued. *See generally In re O'Farrell*, 853 F.2d 894, 903, 7 U.S.P.Q.2d 1673, 1681 (Fed. Cir. 1988) (defining obvious-to-try as when prior art gives "only general guidance as to the particular form of the claimed invention or how to achieve it").

In re Lilly & Co., 14 U.S.P.Q.2d 1741, 1743 (Fed. Cir. 1990). Applicant believes that *Lindell* and *Lilly*, are directly on point with respect to the facts of the instant case.

Applicant concedes, and the Specification recites, that it was known that that derivatives of 3-phenylacetylamino-2,6-piperidinedione could inhibit, *in vitro*, the enzymatic activity of one of the proteins involved in the synthesis of cholesterol. However, this information can only be viewed as sufficient to, in the language of *Lilly* cited above, "pique the scientist's curiosity, such that further investigation might be done as a result of the disclosure". As held by the court in

Lindell this level of disclosure is insufficient to render obvious the result of subsequent "well planned" research.

Thus, in the instant case, Applicant knew that that derivatives of 3-phenylacetylamino-2,6-piperidinedione had been shown to inhibit, *in vitro*, the activity of a single enzyme known to be involved in the biosynthesis of cholesterol (specifically, as discussed in part "**B.**", *infra*, Applicant knew that it had been reported that phenylacetate and some of is derivatives could inhibit the *in vitro* formation of (*see*, ., M. Costillo *et al.* "Inhibition of chick brain cholesterogenic enzymes by phenyl and phenolic derivatives of phenylalanine", *Neurochem. Int.* vol. (1991)18:171-174., Exhibit C)). Applicant's previous work had demonstrated that phenylacetic acid is a breakdown product of 3-phenylacetateylamino-2,6-piperidinedione. However, **no one** had demonstrated that 3-phenylacetateylamino-2,6-piperidinedione could block the formation of iso-pentenylpyrophosphate from 5-pyrophosphomevalonate. This knowledge motivated Applicant to speculate that 3-phenylacetateylamino-2,6-piperidinedione and/or its derivatives might be used as part of a therapy to help treat hypercholesterolemia and/or hypertriglyceridemia. Therefore, Applicant commenced a course of "well-planned research" to find out whether "*these compounds may lower serum cholesterol levels*." This endeavor included experiments to determine which, if any, of the described compounds could be used safely and effectively as part of a treatment to ameliorate one or both of these conditions, in afflicted patients. Subsequently, through the use of carefully planned and monitored experiments, such as the one described at pages 16 and 17 of the Specification, Applicant discovered that the claimed compounds are efficacious for reducing elevated cholesterol and/or triglyceride levels.

In view of the reasoning and case-law presented above, Applicant believes that the Examiner's final rejection of claims 1-4, 8, and 19-23, under 35 U.S.C. § 103(a), as being

obvious over Applicant's alleged admission taken in view of Hendry *et al.* fails to establish a *prima facie* case of obviousness and should therefore be reversed. Furthermore, Applicant believes that the rejection should be reversed regardless of whether or not the Examiner's characterization of the alleged admission is finally determined to be accurate.

B. Status of statement alleged to be an admission.

The Examiner has alleged that page 3, lines 18-23 of the instant Specification, which recites:

> [i]t has been known for some time that compounds such as 3-phenylacetylamino-2,6-piperidinedione and its hydrolysis products, such as phenylacetic acid, and salts, precursors, and analogs thereof (together, "3-phenylacetylamino-2,6-piperidinedione and its derivatives"), can block the formation of iso-pentenylpyrophosphate from 5-pyrophosphomevalonate, a reaction in the pathway of cholesterol biosynthesis; as a result, these compounds may lower serum cholesterol levels.

constitutes and admission that it was known in the prior art that the compounds recited in the pending claims "may lower serum cholesterol level." *See*, paragraph at the bottom of page 4 of the Office Action mailed January 31, 2001. In response to this allegation Applicant points out that the sentences which immediately follows the above quotation recite: "*[t]herefore, it was desirable to determine which, if any, of 3-phenylacetylamino-2,6-piperidinedione and its derivatives can lower serum cholesterol levels, and thus form the basis of a pharmaceutical composition useful for treating or preventing hypercholesterolemia. Derivatives of 3-phenylacetylamino-2,6-piperidinedione that exhibit such activity are disclosed herein.*" These sentences makes it clear that the recitation that "these compounds may lower serum cholesterol levels" is merely speculative and was the basis for the Applicant's research that led to the claimed invention. Moreover, the phrase "*as a result, these compounds may lower serum cholesterol levels*" (*Id.*) is set-off from the remainder of the sentence by a semi-colon. Thus it is

Applicant's position that both the statement's punctuation and its context indicate a distinct demarcation between what was known in the related art and what was known only by the Inventor.

Furthermore, while the specification does recite the phrase "[i]t has been known for some time" (*Id.*.), the specification provides no suggestion as to who possessed this information. It is Applicant's position that this phrase--which is part of the paragraph providing the transition between the "Description of the Related Art" and "Summary of the Invention" sections--was only meant to convey that it was known in the "related" art that the phenylacetic acid, and analogs thereof, could block enzymatic activity *in vitro* (*see*, page 5 of the response filed April 24, 2001). Consequently, Applicant asserts that the ability of the compounds identified in the present claims to lower serum cholesterol levels was known only to Applicant and that this ability was discovered as a result of the research which led to the instantly claimed invention (*see* paragraph bridging pages 5 and 6 of the response filed April 24, 2001).

In reply to assertions, analogous to those above, Examiner alleged that:

> Applicant's admissions do not address the presence or absence of a host, neither is there any mention if *in vitro* versus *in vivo* application or employment of these compounds. Therefore, applicant's remarks regarding mere *in vitro* enzyme blockage in the prior art as represented by his admissions in the specification are unpersuasive.

See, Office Action dated August 14, 2001, page 5, first paragraph.

While Applicant concedes that the passage may not explicitly recite *in vitro* versus *in vivo* use; the fact that this text refers to *in vitro* use is undeniably implicit in a plain language reading of the passage, when read in its context. Furthermore, Applicant believes that the characterization that the admission is part of the prior art is improper. Nowhere, does the Specification characterize the phrase "*as a result, these compounds may lower serum cholesterol levels*" as being part of the "prior art". Additionally, the Examiner's characterization runs

contrary to current case law precedent. In *In re Nomiya*, 509 F.2d 566, 184 U.S.P.Q.2d 607, 610 the court found that Figures, in the Application, which were specifically labeled as "Prior Art" constituted an admission that what was pictured was prior art. In contrast, in the present case, Applicant has made no such admission. Nowhere has Applicant characterized the possibility that the compounds described in the claims could lower serum cholesterol levels as being part of the "prior art".

In addition, the Court of Appeals of the Federal Circuit has specifically held that "a patent applicant's statement of the purpose of the work is not prior art." *In re Dow Chemical Co.*, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). It is Applicant's position that the Examiner's rejection, which depends on the alleged admission, has precisely the effect proscribed by *In re Dow Chemical Co.* Accordingly, this characterization is improper and cannot, legitimately, be used to support a rejection of the appealed claims under 35 U.S.C. § 103(a).

As further evidence in support of Applicant position, submitted herewith, as Exhibit C, is the scientific article which is summarized by the alleged admission (*i.e.*, M. Costillo *et al.* "Inhibition of chick brain cholesterogenic enzymes by phenyl and phenolic derivatives of phenylalanine", *Neurochem. Int.* vol. (1991)18:171-174, referred to hereinafter as "Costillo *et al.*"). Significantly, Costillo *et al.* describes only the use of phenylacetate--and a limited number of related phenyl and phenolic compounds--to cause the *in vitro* inhibition of mevalonate 5-pyrophosphate (MVAPP)[1] decarboxylase derived from chick brain or liver. *See*, Costillo *et al.* page 171, right column. There is nothing in Costillo *et al.* which teaches or suggest that the compounds described in the present claims may be used to lower serum cholesterol levels in patients afflicted with hypercholesterolemia.

[1] MVAPP is the enzyme which catalyzes the conversion of 5-pyrophosphomevalonate to iso-pentenylpyrophosphate.

In view of Exhibit C and the arguments presented above, Applicant contends that the Examiner's characterization of the disputed phrase has been unequivocally refuted. There is no evidence or case law which supports the Examiner's position that the disputed phrase constitutes an admission which could be interpreted to mean that it was known in the prior art that "these compounds may lower serum cholesterol levels."

Furthermore, regardless of the final determination regarding the meaning of the disputed passage, it is clear that the Applicant has not conceded that it was known that cholesterol synthesis is inhibited or that serum cholesterol levels are lowered by treatment with the compounds described in the claims.

In summary, it is Applicants position that when read in context, the alleged admission cannot reasonably be construed to mean that 3-phenylacetylamino-2,6-piperidinedione and/or its derivatives was/were known to block the enzymatic conversion of 5-pyrophosphonmevalonate to iso-pentenylpyrophosphate *in vivo*; nor that these compounds would be useful to treat hypercholesterolemia or hypertriglyceridemia. Accordingly, the rejection of the claims under 35 U.S.C. § 103(a) is predicated on an improper characterization of the Applicant's Specification and should be reversed.

C. Disclosure of *In vitro* activity does not render *In vivo* function obvious

As discussed above, it is Applicant's position that the prior art describes only that derivatives of 3-phenylacetylamino-2,6-piperidinedione can inhibit, *in vitro*, the function of one specific enzyme, which enzyme is known to be involved in the biosynthesis of cholesterol. As noted, *supra*, the Examiner contends that the Applicant has admitted that these compounds are known to inhibit this enzymatic step *in vivo* (a position which Applicant adamantly disputes). Nevertheless, the Examiner has stated that:

> even if applicant's admission in the specification regarding the prior art were limited to *in vitro* enzyme activity, it is well known in the pharmaceutical art that the purpose of *in vitro* experimentation with pharmaceutical activities is to ultimately administer the active composition *in vivo* to an affected host/patient for some sort of therapy. This sort of testing is conventional in the pharmaceutical art...Therefore one of ordinary skill in the art would have reasonably believed that 3-phenylacetylamino-2,6-piperidinedione and its derivatives known to block a specific step in the cholesterol biosynthetic pathway and in turn block cholesterol synthesis resulting in lowering of blood cholesterol levels . . . .

See August 14, 2001 Office Action, page 5. Applicant traverses.

Applicant unreservedly agrees with Examiner's statement that the "purpose of *in vitro* experimentation with pharmaceutical activities is to ultimately administer the active composition *in vivo* to an affected host/patient for some sort of therapy." *Id* (emphasis added). However, Applicant, asserts that this very language describes the quintessential "obvious to try" paradigm. Applicant, contends that by teaching only that a certain compound has *in vitro* inhibitory activity against a single enzyme in the cholesterol biosynthetic pathway, a prior art reference cannot do more than pique a scientist's curiosity and motivate further investigation, which is the definition of "obvious-to-try" provided by *In re Lilly*. It is well known in the pharmaceutical arts that *in vitro* identification of a promising "lead" compound is merely the beginning of the long and expensive process of developing a safe and efficacious treatment. Furthermore, it is also well known that the overwhelming majority of "lead" compounds fail to yield desirable results in subsequent animal and/or clinical trials.

Consequently, Applicant contends that the Examiner has, at most, merely asserted that the claimed invention is obvious. The Examiner has provided no evidence in support of this assertion. Thus, the Examiner has provided insufficient grounds for rejection, given that the Court of Appeals of the Federal Circuit recently held that:

> [a]s an administrative tribunal the Board clearly has expertise in the subject matter over which it exercises jurisdiction. This expertise may provide sufficient support for conclusions as to the peripheral issues. With respect to core factual findings

> in a determination of patentability, however, the Board cannot simply reach conclusions based on its own understanding or experience--or on its assessment of what would be basic knowledge or common sense. Rather the Board must point to some concrete evidence in the record in support of these findings.

In re Zurko, 258 F.3d 1379, 1389, 59 U.S.P.Q.2d 1693 (Fed. Cir. 2001). Consequently, Applicant contends that, contrary to the holding in *Zurko*, the Examiner has provided no evidence, which teaches or suggests that a known *in vitro* function for a given compound renders it obvious that the same compound will have any specific effect *in vivo* or that a treatment based on the compound would have a reasonable expectation of success. Instead, the Examiner has merely indicated that it is common to use *in vitro* results as motivation for further investigation.

Furthermore, Applicant argues that even if the Examiner's position were conceded (*i.e.*, that the Specification provides an admission that it was known that 3-phenylacetylamino-2,6-piperidinedione and/or its derivatives *may* inhibit the enzymatic conversion of iso-pentenylpyrophosphate to 5-pyrophosphonmevalonate *in vivo*), this would constitute evidence only sufficient to render the claimed invention "obvious-to-try". As noted in the biochemistry text section cited above, the metabolic processes which contribute to and govern serum cholesterol levels are complex and do not depend solely on cholesterol biosynthesis rates (*e.g.*, cholesterol levels also depend on the rate of clearance by liver enzymes). Thus, even if the Specification is, improperly, construed as an admission that it was know that the compounds, used in the claimed methods, may inhibit cholesterol synthesis *in vivo*, this still only renders the claims "obvious-to-try." Taken either alone, or in conjunction with the other cited art, such an admission does not provide a "reasonable expectation" that the claimed methods would succeed. Furthermore, contrary to the requirement set out in *Zurko*, the Examiner has provided no evidence, beyond mere assertion, that it would have been obvious to succeed.

For the reasons argued above Applicant asserts that, regardless of how the Specification's alleged admission is construed, the cited art does not render the claimed invention obvious. Consequently, the Examiner's objection is untenable and should be reversed.

***

IX. CONCLUSION

For all of the reasons explained, above, Applicant contends that the Examiner has not established a *prima facie* case for obviousness of the appealed claims with respect to the cited art. The Examiner has both grossly misinterpreted the Specification and then used that misinterpretation to reject the appealed claims. The Examiner has thus stacked unsupported factual inference upon unsupported factual inference. Firstly, without any evidence or rationale to support her position, the Examiner has inferred, despite Applicant's assertions to the contrary, that one of ordinary skill would have interpreted the Specification as comprising an admission that it was known that the compounds described in the claimed methods would inhibit cholesterol synthesis *in vivo*. *See*, August 14, 2001 Office Action. Secondly, based on the first inference, the Examiner has inferred that one of ordinary skill in the art would reasonably believed that the claimed invention would be effective.

Applicant contends that, contrary to the standard established in *Zurko*, the Examiner has failed to provide evidentiary support for either of the described inferences. Thus, the Examiner has failed to establish a *prima facie* case of obviousness.

Therefore, Applicant believes that the rejection of the appealed claims, as being obvious under 35 U.S.C. § 103(a), is improper and should be reversed.

Respectfully submitted,

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Date: March 1, 2002

APPENDIX A

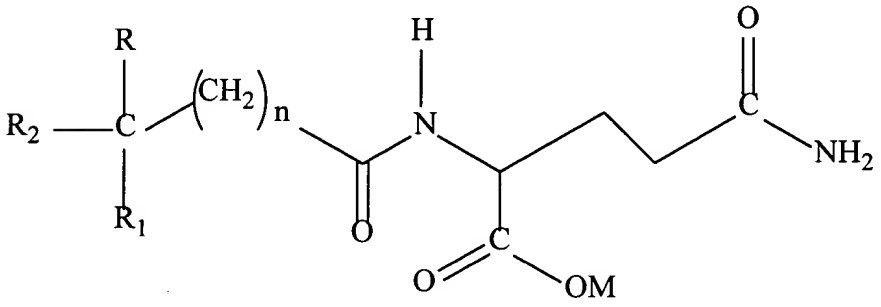
(PENDING CLAIMS)

WHAT IS CLAIMED IS:

1. (Twice Amended) A method for the treatment or inhibition of hypercholesterolemia or hypertriglyceridemia in an affected patient, comprising the step of:

administering to the patient a composition comprising a therapeutically-effective amount of a compound of either Formula I:

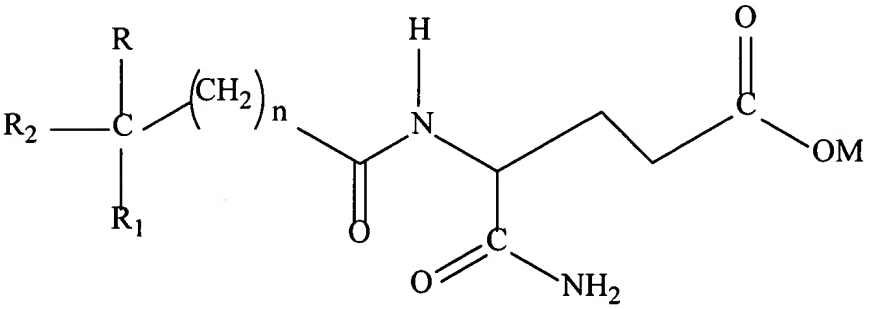
a) Formula I



wherein R and R_1 are independently selected from the group consisting of H, lower alkoxy (C_{1-6}), or lower alkyl (C_{1-6}); R_2 is selected from the group consisting of aryl (C_{6-12}) and substituted aryl; M is hydrogen, sodium, potassium, ammonium, diethanolamine, cyclohexylamine, a naturally-occurring amino acid of MW less than 500 kD, lower alkyl (C_{1-6}), cycloalkyl, or aryl (C_{6-12}); and n is 0-5;

b) Formula III:

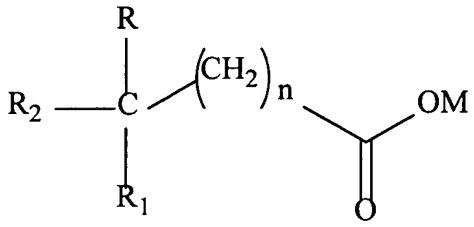
Formula III



wherein R and R_1 are independently selected from the group consisting of H, lower alkoxy (C_{1-6}), or lower alkyl (C_{1-6}); R_2 is selected from the group consisting of aryl (C_{6-12}) and substituted aryl; M is hydrogen, sodium, potassium, ammonium, diethanolamine, cyclohexylamine, a naturally-occurring amino acid of MW less than 500 kD, lower alkyl (C_{1-6}), cycloalkyl, or aryl (C_{6-12}); and n is 0-5;

c) Formula IV:

Formula IV

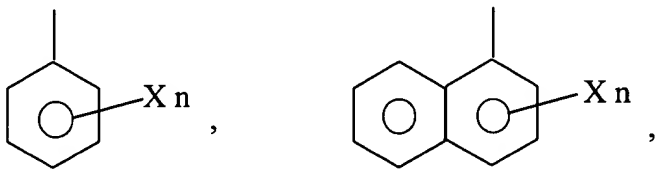


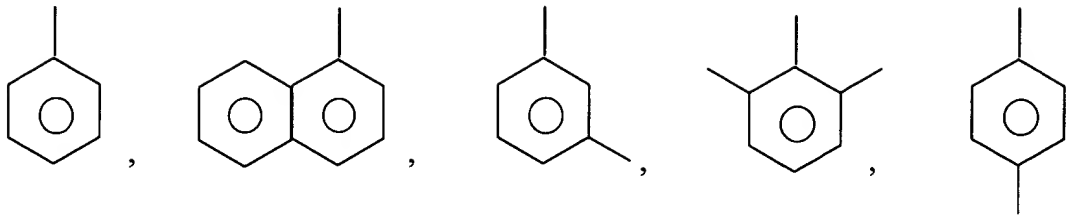
wherein R and R_1 are independently selected from the group consisting of H, lower alkoxy (C_{1-6}), or lower alkyl (C_{1-6}); R_2 is selected from the group consisting of aryl (C_{6-12}) and substituted aryl; M is hydrogen, sodium, potassium, ammonium, diethanolamine, cyclohexylamine, a naturally-occurring amino acid of MW less than 500 kD, lower alkyl (C_{1-6}), cycloalkyl, or aryl (C_{6-12}); and n is 0-5; or,

d) any combination thereof.

2. The method of claim 1, wherein in said composition M is hydrogen, potassium or sodium; n is 0-2; R and R_1 are independently selected from the group consisting of H and C_3H_7; R_1 is selected from the group consisting of H, CH_3, CH_3-O-, C_2H_5, and C_3H_7; and R_2 is an aryl (C_{6-12}) or a substituted aryl selected from the group consisting of Formula II:

Formula II





, wherein X is a halogen, lower alkyl (C_{1-6}), lower alkoxy (C_{1-6}), cycloalkyl, cycloalkoxy, aryl (C_{6-12}), substituted aryl or hydroxy and n is 0, 1, 2, 3, or 4.

3. The method of claim 2, wherein said therapeutically-effective amount is from 20 mg/kg/day to 2500 mg/kg/day.

4. The method of claim 1, wherein said composition further comprises at least one pharmaceutically-acceptable carrier, diluent, or excipient.

8. The method of claim 2, wherein said composition further comprises at least one pharmaceutically-active carrier, diluent, or excipient.

16. The method of claim 2, wherein said composition comprises an effective amount of phenylbutyric acid, phenylbutylglutamine, isophenylbutylglutamine or pharmaceutically acceptable salts thereof.

19. (New) The method of claim 1, wherein the compound of Formula I is the sodium salt of phenylacetylglutamine, the compound of Formula III is the sodium salt of phenylacetylisoglutamine, and the compound of Formula IV is the sodium salt of phenylacetate.

20. The method of claim 1, wherein said therapeutically-effective amount is from 20 mg/kg/day to 2500 mg/kg/day.

21. The method of claim 1, wherein the compound of Formula IV is phenylacetic acid, a pharmaceutically acceptable salt of phenylacetic acid, or mixtures thereof.

22. The method of claim 1, wherein the compound of Formula I is phenylacetylglutamine, a pharmaceutically acceptable salt of phenylacetylglutamine, or mixtures thereof.

23. The method of claim 1, wherein the compound of Formula III is phenylacetylisoglutamine, a pharmaceutically acceptable salt of phenylacetylisoglutamine, or mixtures thereof.

APPENDIX B

BIOCHEMISTRY

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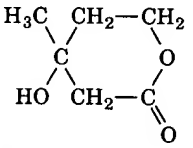
HMG-CoA reductase and [cAMP] places HMG-CoA reductase activity and thus cholesterol biosynthesis under hormonal control. Insulin, which decreases [cAMP], stimulates cholesterol biosynthesis. Glucagon, which increases [cAMP], inhibits it. These effects are consistent with the actions of insulin and glucagon on other metabolic pathways in liver, such as glycolysis, glycogen synthesis and breakdown, gluconeogenesis, and fatty acid biosynthesis and breakdown (Sections 17-3F and 23-5). The modification/demodification system is also influenced by the presence of LDL–cholesterol and **mevalonolactone** (an internal ester of mevalonate that is hydrolyzed to mevalonate and metabolized in the cell),



Mevalonolactone

which inhibit HMG-CoA reductase activity by stimulating its phosphorylation. Research is actively underway to determine how this process occurs.

LDL Receptor Activity Controls Cholesterol Homeostasis

LDL receptors clearly play an important role in the maintenance of plasma LDL–cholesterol levels. In normal individuals, about one half of the IDL formed from the VLDL reenters the liver through LDL receptor–mediated endocytosis (IDL and EDL both contain apolipoproteins that specifically bind to the LDL receptor; Section 11-4B). The remaining IDL are converted to LDL (Fig. 23-49*a*). *The serum concentration of LDL therefore depends on the rate that liver removes IDL from the circulation which, in turn, depends on the number of functioning LDL receptors on the liver cell surface.*

High blood cholesterol **(hypercholesterolemia)**, which results from the overproduction and/or underutilization of LDL, is known to be caused by two metabolic irregularities: (1) the genetic disease **familial hypercholesterolemia (FH)**; or (2) the consumption of a

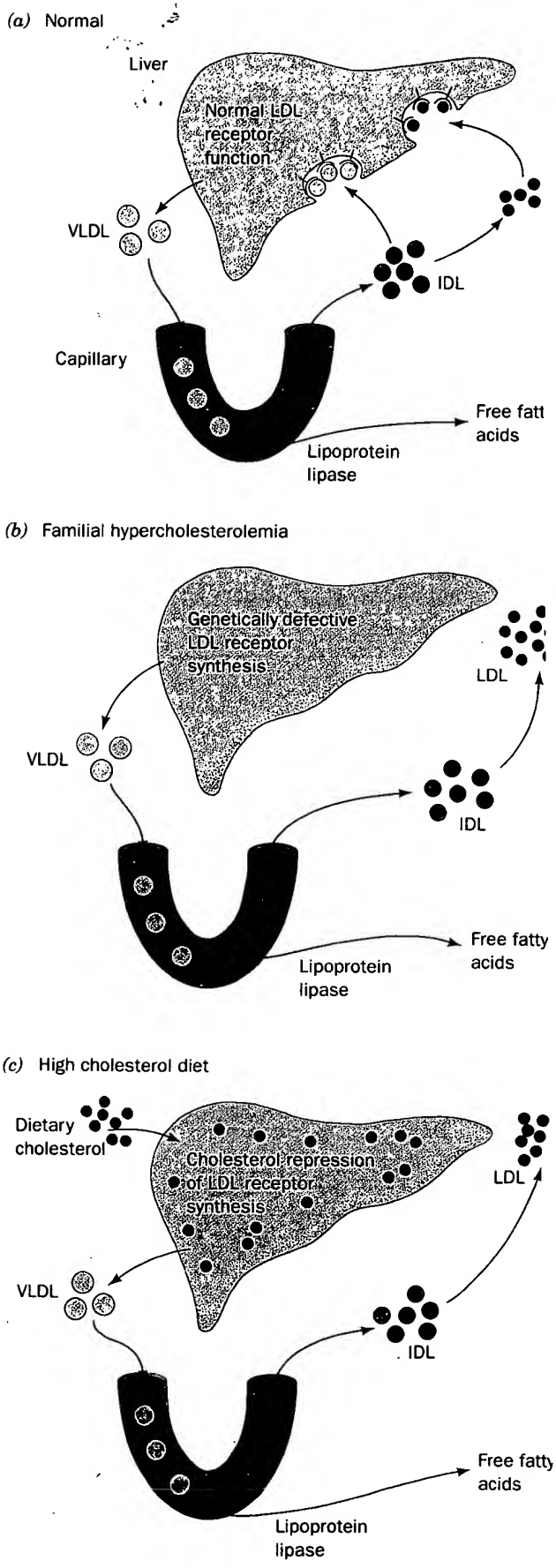


Figure 23-49
Liver LDL receptors control plasma LDL production and uptake. (*a*) In normal human subjects, VLDL is secreted by the liver and converted to IDL in the capillaries of the peripheral tissues. About one half of the plasma IDL particles bind to the LDL receptor and are taken up by the liver. The remainder are converted to LDL at the peripheral tissues. (*b*) In individuals with familial hypercholesterolemia (FH), liver LDL receptors are diminished or eliminated because of a genetic defect. (*c*) In normal individuals who ingest a high cholesterol diet, the liver is filled with cholesterol, which represses the rate of LDL receptor production. Receptor deficiency, whether of genetic or dietary cause, raises the plasma LDL level by increasing the rate of LDL production and decreasing the rate of LDL uptake. [After Goldstein J. L. and Brown, M. S., *J. Lipid Res.* **25**, 1457 (1984).]

high cholesterol diet. FH is a dominant genetic defect that results in a deficiency of functional LDL receptors (Section 11-4C). Homozygotes for this disorder lack functional LDL receptors so that their cells can absorb neither IDL nor LDL by receptor-mediated endocytosis. The increased concentration of IDL in the bloodstream leads to a corresponding increase in LDL which is, of course, underutilized since it cannot be taken up by the cells (Fig. 23-49*b*). FH homozygotes therefore have plasma LDL-cholesterol levels three to five times higher than average. FH heterozygotes, which are far more common, have about one half of the normal number of functional LDL receptors and plasma LDL-cholesterol levels of about twice the average.

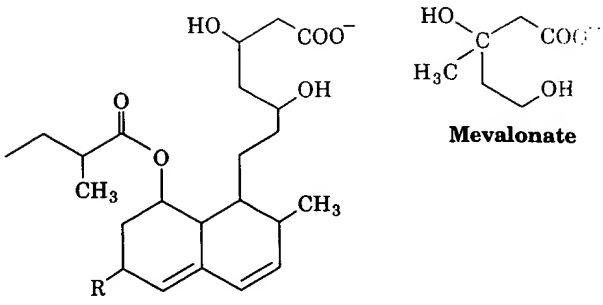
The ingestion of a high cholesterol diet has an effect similar, although not as extreme, as FH (Fig. 23-49c). Excessive dietary cholesterol enters the liver cells in chylomicron remnants and represses the synthesis of LDL-receptor protein. The resulting insufficiency of LDL receptors on the liver cell surface has consequences similar to those of FH.

LDL receptor deficiency, whether of genetic or dietary origin, raises the LDL level by two mechanisms: (1) increased LDL production resulting from decreased IDL uptake; and (2) decreased LDL uptake. Two strategies for reversing these conditions (besides maintaining a low cholesterol diet) have been tested:

1. *Ingestion of resins that bind bile acids thereby preventing their intestinal absorption.* Bile acids are normally efficiently recycled by the liver (Section 23-6C). Elimination of resin-bound cholesterol in the feces forces the liver to convert more cholesterol to bile acids than normal. The consequent decrease in the serum cholesterol concentration induces synthesis of LDL receptors (of course, not in FH homozygotes). Unfortunately, the decreased serum cholesterol level also induces the synthesis of HMG-CoA reductase, which increases the rate of cholesterol biosynthesis. Ingestion of bile acid-binding resins therefore provides only a 15 to 20% drop in serum cholesterol levels.

2. *Treatment with competitive inhibitors of HMG-CoA reductase, notably the fungal products* ***compactin*** *and* ***lovastatin*** *(also called* ***mevinolin****; Fig. 23-50), so as to decrease the rate of cholesterol biosynthesis.* Indeed, lovastatin has recently received clinical approval for the treatment of hypercholesterolemia. The resulting decreased cholesterol supply is again met by induction of LDL receptors and HMG-CoA reductase. Lovastatin-treated FH heterozygotes nevertheless routinely show a serum cholesterol decrease of 30%.

The combined use of these agents, moreover, results in a clinically dramatic 50 to 60% decrease in serum cholesterol levels.



Mevalonate

R = H **Compactin**
R = CH_3 **Lovastatin (mevinolin)**

Figure 23-50
Compactin and lovastatin, two potent inhibitors of HMG-CoA reductase. The structure of mevalonate is shown for comparison.

C. Cholesterol Utilization

Cholesterol is the precursor of ***steroid hormones*** *and bile acids.* Steroid hormones are grouped into five categories: ***progestins, glucocorticoids, mineralocorticoids, androgens, and estrogens.*** These hormones, as described in Section 34-4A, mediate a wide variety of vital physiological functions. All contain the four-ring structure of the sterol nucleus and are remarkably similar in structure, considering the enormous differences in their physiological effects. A simplified biosynthetic scheme (Fig. 23-51) indicates their structural similarities and differences. We shall not discuss the details of these pathways.

The quantitatively most important pathway for the excretion of cholesterol in mammals is the formation of bile acids (also called ***bile salts****).* The major bile acids, **cholic acid** and **chenodeoxycholic acid,** are synthesized in the liver and secreted as glycine or **taurine** conjugates (Fig. 23-52) into the gallbladder. From there, they are secreted into the small intestine where they act as emulsifying agents in the digestion and absorption of fats and fat-soluble vitamins (Section 23-1). An efficient recycling system allows the bile acids to reenter the bloodstream and return to the liver for reuse several times each day. The $< 1\ g \cdot day^{-1}$ of bile acids that normally escape this recycling system are further metabolized by microorganisms in the large intestine and excreted. *This is the body's only route for cholesterol excretion.*

Comparison of the structures of cholesterol and the bile acids (Figs. 23-34 and 23-52) indicates that biosynthesis of bile acids from cholesterol involves (1) saturation of the 5,6-double bond, (2) epimerization of the 3β-OH group, (3) introduction of OH groups into the 7α and 12α positions, (4) oxidation of C(24) to a carboxylate, and (5) conjugation of this side chain carboxylate with glycine or taurine.

APPENDIX C

Neurochem. Int. Vol. 18, No. 2, pp. 171–174, 1991
Printed in Great Britain

0197-0186/91 $3.00 + 0.00

INHIBITION OF CHICK BRAIN CHOLESTEROGENIC ENZYMES BY PHENYL AND PHENOLIC DERIVATIVES OF PHENYLALANINE

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(Received 10 January 1990; accepted 9 April 1990)

Abstract—Phenylalanine and its phenyl metabolites produced a clear inhibition of chick brain mevalonate 5-pyrophosphate decarboxylase, while mevalonate kinase and mevalonate 5-phosphate kinase were not significantly affected. Phenolic derivatives produced a similar or higher inhibition than that found in the presence of phenyl metabolites. The inhibition was progressive with increasing concentrations of inhibitors (1.25–5.00 mM). Phenylpyruvate and *p*-hydroxyphenyl-lactate were the most potent inhibitors of decarboxylase activity. Simultaneous supplementation of each metabolite at 0.25 mM concentration produced a considerable inhibition of brain decarboxylase and 3-hydroxy-3-methylglutaryl-CoA reductase. At our knowledge this is the first report on the *in vitro* inhibition of both brain regulatory enzymes of cholesterogenesis in phenylketonuric-like conditions.

It is generally accepted that phenylketonuria (PKU) is characterized by the absence or deficiency of hepatic phenylalanine hydroxylase (Knox, 1972). Thus, the conversion of phenylalanine to tyrosine is blocked, resulting in high tissue levels of phenylalanine and secondary disturbances in its metabolism, which are thought to be responsible for more or less severe brain damage. Literature mentions a number of theories about the cause of this brain damage, some of them related with an impaired myelination (Crome and Pare, 1960). It has been suggested that lipid metabolism is abnormal in PKU conditions (Menkes, 1967). Several studies have demonstrated a reduction in the cholesterol levels of brain of PKU subject (Crome *et al.*, 1962; Gerstl *et al.*, 1967) although other authors have not been able to confirm these findings (Foote *et al.*, 1965).

Active synthesis of sterols is an important feature of the developing central nervous system, coinciding with the process of myelination (Wells and Dittmer, 1967; Cuzner and Davison, 1968; Bass *et al.*, 1970). The few studies concerned with the enzymatic regulation of cholesterogenesis in the developing brain have emphasized the role of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (EC 1.1.1.34), which is known to be a major rate-limiting enzyme in this biosynthetic pathway in other tissues (Siperstein, 1970; Schroepfer, 1981), although the existence of secondary sites beyond mevalonic acid (MVA) formation has also been proposed (Marco *et al.*, 1983, 1986). In a previous paper (Marco *et al.*, 1984), we have reported the induction of experimental PKU-like conditions in chick embryo between 10–20 days of incubation by daily injection of phenylalanine and α-methylphenylalanine, an inhibitor of hydroxylase without toxic effects on animal growth (Greengard *et al.*, 1976). In these conditions, we have found that only brain mevalonate 5-pyrophosphate (MVAPP) decarboxylase (EC 4.1.1.33) activity was significantly inhibited at the onset of myelination (Alejandre *et al.*, 1983). However, when experimental hyperphenylalaninemia was induced in neonatal chick (Castillo *et al.*, 1988a), a significant inhibition of brain and liver HMG-CoA reductase and MVAPP decarboxylase activities was observed (Castillo *et al.*, 1988b), corroborating the interference of hyperphenylalaninemia with the cholesterogenic pathway. Because of this, in this work we have studied the effect of phenylalanine and its derivatives accumulated in PKU on chick brain cholesterogenic enzymes, showing for the first time an *in vitro* inhibition of both reductase and decarboxylase cerebral activities by these metabolites.

EXPERIMENTAL PROCEDURES

[5-^{14}C]HMG-CoA, [2-^{3}H]MVA lactone and [1-^{14}C]MVA lactone were supplied by Amersham International, Amersham, U.K. Phenylalanine and its metabolites were

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purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. All other reagents were analytical grade.

Newborn White Leghorn male chicks (*Gallus domesticus*) were obtained from a commercial hatchery and maintained fed *ad libitum* on a commercial diet (Sanders A-00) in a chamber with a light cycle from 09.00 to 21.00 h and controlled temperature (28°C). In our experiments, 15-day-old chicks were used. Chicks were killed by decapitation at the same time every day (10 a.m.). Enzyme preparations were obtained from brains as previously described for measurement of HMG-CoA reductase (Ramirez *et al.*, 1981) and MVA activating enzymes (Gonzalez-Pacanowska *et al.*, 1981). Protein contents were determined by the method of Lowry *et al.* (1951). Enzyme preparations were preincubated for 10 min with each metabolite tested. HMG-CoA reductase activity was measured essentially as described by Shapiro *et al.* (1974) using [2-^{3}H]MVA lactone as an internal standard, with modifications described by Alejandre *et al.* (1981). Mevalonate phosphorylation and decarboxylation were measured as previously described (Gonzalez-Pacanowska *et al.*, 1985). Mevalonate 5-phosphate (MVAP) and MVAPP were identified by paper chromatography and radioactivity was measured by liquid scintillation (Marco *et al.*, 1983). Total MVA kinase activity (EC 2.7.1.36) was measured by adding the amounts of MVAP and MVAPP formed during the reaction to the amount of CO_2 formed. Similarly, MVAP kinase (EC 2.7.4.2) activity was measured by adding the amounts of MVAPP and CO_2 formed. MVAPP decarboxylase activity was considered as the amount of CO_2 formed. All the specific activities were expressed as nmol/30 min per mg protein.

RESULTS

The *in vitro* MVA phosphorylation and decarboxylation by chick brain 105.000 *g* supernatants were studied in the presence of phenylalanine and its metabolites added at different concentrations. Table 1 shows that phenylalanine and its phenyl derivatives produced a clear inhibition of MVAPP decarboxylase while the specific activities of MVA kinase and MVAP kinase were not significantly affected in these conditions. Therefore, differences observed in $^{14}CO_2$ production from [1-^{14}C] MVA could be only related with MVAPP decarboxylase. In all the cases studied, inhibition of decarboxylase activity was progressive with increasing concentrations of inhibitors (1.25–5.00 mM). Phenylalanine and phenylethylamine showed the lesser degree of inhibition.

The effect of some phenolic compounds on brain MVA phosphorylation and decarboxylation was also studied. Results in Table 1 show that *o*- and *p*-hydroxyphenylacetate as well as *p*-hydroxyphenyl-

Table 1. Effect of phenylalanine and different phenyl and phenolic derivatives on chick brain MVAPP decarboxylase

Additions	Concentration (mM)	MVAPP decarboxylase (nmol/30 min/mg protein)	Inhibition (%)
None	—	14.59 ± 0.48	—
Phenylalanine	1.25	14.20 ± 0.60	—
	2.50	11.79 ± 0.61*	19.2
	5.00	10.74 ± 0.90*	26.4
Phenylpyruvate	1.25	8.88 ± 1.10**	39.1
	2.50	7.38 ± 1.00***	49.4
	5.00	4.17 ± 0.60***	71.4
Phenylacetate	1.25	11.46 ± 0.51**	21.4
	2.50	10.46 ± 0.11***	28.3
	5.00	5.88 ± 0.80***	59.7
Phenyl-lactate	1.25	11.32 ± 0.21***	23.4
	2.50	8.01 ± 0.30***	45.1
	5.00	7.08 ± 0.21***	51.5
Phenylethylamine	1.25	12.18 ± 0.52*	16.5
	2.50	11.75 ± 0.51**	19.5
	5.00	10.89 ± 0.62**	25.4
o-Hydroxyphenylacetate	1.25	12.01 ± 0.43**	17.6
	2.50	9.46 ± 0.32***	35.2
	5.00	6.00 ± 0.31***	58.9
p-Hydroxyphenylacetate	1.25	10.14 ± 0.32***	30.5
	2.50	9.08 ± 1.41*	37.8
	5.00	8.43 ± 0.32***	42.2
p-Hydroxyphenyl-lactate	1.25	12.38 ± 0.12**	15.1
	2.50	6.90 ± 0.01***	52.7
	5.00	4.50 ± 0.11***	69.2

Reactions were carried out by incubating the enzyme preparations from 15-day-old chick brain as described in Experimental Procedures in the presence of different compounds at the specified concentration. Results are means ± SEM of four experiments with pools of five animals. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$.

Table 2. Inhibition of chick brain MVAPP decarboxylase and HMG-CoA reductase in PKU-like conditions

	MVAPP decarboxylase		HMG-CoA reductase	
	Specific activity (nmol/30 min/mg protein)	Inhibition (%)	Specific activity (nmol/30 min/mg protein)	Inhibition (%)
Control	12.86 ± 1.98	—	0.77 ± 0.01	—
Experimental conditions	7.44 ± 0.62**	42.2	0.18 ± 0.02*	76.3

In these experimental conditions, reactions were carried out by incubating the enzyme preparations obtained from 15-day-old chick brain as described in Experimental Procedures in the presence of all the phenyl and phenolic compounds previously assayed at 0.25 mM concentration each. Results are means ± SEM of three experiments with pools of five animals. * $P < 0.05$; ** $P < 0.005$.

lactate were inhibitory to MVAPP decarboxylase, while MVA kinase and MVAP kinase were not practically affected at any concentration assayed (1.25–5.00 mM).

Comparison of the degree of MVAPP decarboxylase inhibition at the different conditions assayed is shown in Table 1. At low concentration (1.25 mM), phenylpyruvate was the more potent inhibitor while phenylalanine had no effect on decarboxylase activity. However, at high concentration (5.00 mM) *p*-hydroxyphenyl-lactate showed a similar inhibitory effect to phenylpyruvate, followed by phenylacetate and *o*-hydroxyphenylacetate. The lowest differences between the degree of inhibition observed at different inhibitor concentrations were found in the presence of phenylethylamine.

In view of the inhibition obtained by each phenyl and phenolic compound at 1.25–5.00 mM concentration, we have investigated the effect on the main regulatory cholesterogenic enzymes of a mixture of these metabolites in the same range as that of the hyperphenylalaninemic conditions. Thus, the action of simultaneous supplementation of 0.25 mM inhibitors was tested. Table 2 shows a considerable inhibition of brain MVAPP decarboxylase in these experimental conditions. Likewise, brain HMG-CoA reductase was also strongly inhibited in the same conditions (Table 2).

DISCUSSION

The inhibition of the sterol biosynthesis by phenyl and phenolic compounds had been previously reported in rat liver (Ranganathan and Ramasarma, 1973). The presence of the aromatic ring and the carboxyl group in a molecule appeared to be necessary for the inhibition. The site of inhibition was suggested to be between MVA and isopentenyl pyrophosphate. Different phenyl derivatives also inhibited MVA incorporation into nonsaponifiable lipids in rat brain homogenates and 100,000 *g* supernatants. Phenylpyruvate was the most potent of the inhibitory compounds while phenylalanine *per se* had little effect on mevalonate decarboxylation (Shah *et al.*, 1969).

However, no information is available on the effect of phenylethylamine and different phenolic metabolites also accumulated in brain as a consequence of the disturbances in the conversion of phenylalanine to tyrosine. Our results show that inhibition observed by phenolic compounds was similar to or higher than that found in the presence of phenyl derivatives. It is important to note that MVAPP decarboxylase is the one MVA-activating enzyme that was inhibited by the various compounds tested, corroborating its important role in the regulation of cholesterogenesis.

On the other hand, it has been found that preincubation of enzyme preparations with phenylpyruvate increased the degree of inhibition both in rat liver (Shama Bhat and Ramasarma, 1979) and in chick brain and liver (unpublished results). Hence, the experiments described in this paper were done after preincubation with the inhibitors for 10 min.

In order to study the influence of phenylalanine metabolites in conditions similar to those in experimental hyperphenylalaninemia (Shama Bhat and Ramasarma, 1979; Fulton *et al.*, 1980), incubations were carried out with the simultaneous addition of each metabolite at a lower concentration (0.25 mM) than that tested in previous experiments. To our knowledge, this is the first report on the *in vitro* inhibition of the main regulatory enzymes of cholesterogenesis in these conditions. Our results also show a greater inhibition of HMG-CoA reductase than that of MVAPP decarboxylase, suggesting again a secondary but important regulatory role of decarboxylase in cholesterol synthesis such as that previously described in other physiological and pathological conditions (Jabalquinto and Cardemil, 1981; Marco *et al.*, 1983, 1986; Gonzalez-Pacanowska *et al.*, 1985, 1986; Iglesias *et al.*, 1989).

Acknowledgement—This work was supported in part by a grant from CAICYT (1219-84).

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